Early life stressful events impaired enteric antimicrobial activity and triggered commensal Escherichia coli overgrowth responsible for visceral hypersensitivity in adult mice

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Poster Presentations by Authors from A-Z (alphabetical order)

Characterization of the effects of the antineoplastic drug 5-fluorouracil on gastrointestinal motility and gut wall structure in the rat

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Objective: The antineoplastic drug 5-fluorouracil (5-FU) is used in the clinic for treatment of solid tumours including colon cancer, but it may induce stomatitis, oesophagopharyngitis, anorexia, nausea, vomits, diarrhoea and enteritis. In experimental animals, 5-FU induces mucositis and/or loosen faeces. To our knowledge, a thorough assessment of the effects of 5-FU on gastrointestinal (GI) motility has never been performed in experimental animals. Our aim was to characterise, using non-invasive (radiographic), invasive (charcoal) and *in vitro* (organ bath) methods the effects of 5-FU on GI motility in the rat.
Methods: Adult male Wistar rats (250–400 g, $n = 8$ rats/group) received an intraperitoneal injection of saline or 5-FU (150 mg/kg). In a group of isolated animals, faeces were assessed for the occurrence of diarrhoea; 4 days after, upper gastrointestinal transit was analysed using the charcoal method. In the remaining, grouped animals, 4 days after drug, either whole GI motility was radiographically assessed (Cabezos *et al.*, 2008), or colonic propulsion of faecal pellets was recorded in a horizontal bath and analysed by means of spatio-temporal maps. Finally, gut wall alterations were evaluated on histological cross sections at the same time-point.
Results: Four days after 5-FU, animals showed less weight than their controls, but diarrhoea was not clearly present. In the radiographic analysis, only a slight acceleration was found in the small intestine emptying phase and the size of the faecal pellets was reduced. Neither upper GI transit measured invasively nor faecal pellets propulsion in organ bath was significantly altered. Mucositis was evident in both ileum and colon, but the smooth muscle was barely affected.
Conclusion: The antineoplastic drug 5-FU induces weight loss and intestinal mucositis in the rat, but GI motility and smooth muscle are scarcely affected. Higher doses, associated to gut wall thinning and visceral pain, might induce motility alterations. Studies of the possible long-term chemotherapy-induced alterations in gut function and structure are needed since they might predispose to gut malfunctioning in cancer survivors.

Beyond acid insult: TREM-1 signalling and lipid-receptors contribute to the pathogenesis of reflux oesophagitis

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Objective: Because the pathophysiology of GORD is not completely understood, available drugs target only one or more of the underlying mechanisms but none are fully effective in all patients. Identifying novel underlying mechanisms may pave the way to develop more effective therapies.
Methods: A surgical model of sub-chronic reflux oesophagitis in Wistar rats was used. Animals were treated with omeprazole (PPI with additional anti-inflammatory activity) or STW5 (herbal preparation of established efficacy in gastro-intestinal disorders) for 7 days before and 10 days after surgery. Histological, proteomic and transcriptomic methods were applied to identify reflux induced changes and treatment responses in oesophagi.
Results: Both test drugs reduced reflux-induced macroscopic and microscopic lesions of the oesophagi as well as most of the measured pro-inflammatory cytokines. Proteomic and transcriptomic analysis identified CINC1-3, MIP-1/3α, MIG, RANTES and IL-1β as highly relevant mediators in GORD. Other highly regulated genes were those of IL-6, CCL3, CCL7 and LOX-1. Many affected cyto-/chemokines were involved in the TREM-1 signalling pathway. The fatty acid receptor GPR84 was highly up-regulated in oesophagitis but down-regulated by both drugs. This was confirmed by Western blot and immune-histochemical staining, as well as in normal human oesophageal squamous cells (HET-1A), showing for the first time expression of this receptor in oesophageal tissue and its possible involvement in GORD.
Conclusion: STW5 and omeprazole target a broad spectrum of molecules involved in immunological and inflammatory processes, of which IL-8 (CINC1-3), TREM-1 pathway, LOX-1 and GPR84 are proposed to be very promising novel targets for the treatment of GORD.

Does frequency of restless leg syndrome and poor sleep quality increase with age in irritable bowel syndrome?

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Objective: Irritable bowel syndrome (IBS) is a common gastrointestinal disease which leads to a decrease in quality of life. Restless leg syndrome (RLS) and poor sleep quality (PSQ) incidence is known to increase in IBS. In this study, we aimed to investigate the prevalence and association of RLS and PSQ in young population.
Methods: A total of 112 IBS patients (46 constipation predominant IBS, 17 diarrhea predominant IBS, 23 mixed IBS, 26 unsubtyped IBS) and 106 healthy controls were included in the study. The Pittsburgh Sleep Quality Index (PSQI) and the 2012 revised diagnostic

investigation of asymptomatic control subjects for calculating mean and two standard deviation above mean. However, today there are not universally accepted norms.
Methods: To assess the feasibility of utilizing an 200-min study we investigated patients with/without GERD referred for 24-h MII-pH-monitoring. Then we applied Receiver Operating Characteristic (ROC)-curve to establish thresholds of abnormal reflux. Patients & Methods: Patients were divided on 2 groups: 1st [none-rosive GERD] - 6 women and 9 men, mean age (±SEM) of 44.8 ± 4.2 years old; 2nd [Functional dyspepsia] - 6 women and 7 men, mean age 49.2 ± 3.9 years old We separately analyzed the data from the 200 min of the study and the data from the entire 24-h period. The test period included 45 min. before and 140 min. after provocative standardized breakfast (507 kCal).
Results: The correlation between the results obtained with each analysis was excellent: for acid reflux - $r = 0.88$ (CI 95% 0.76–0.95); for total reflux - $r = 082$ (CI 95% 0.64–0.92). For fraction time with pH <4 the correlation was worse - $r = 0.69$ (CI 95% 0.41–0.85; $p < 0.0001$). The normal value of number reflux was calculated by means of ROC-curve for detecting GERD. The abnormal reflux was considered when acid reflux are >6 episodes for 200-min and >40 for 24-h period (AUC 0.85 and 0.82 resp.), total reflux - >17 episodes for 200-min and >74 for 24-h period (AUC 0.79 for both).
Conclusion: Our results show that the 200-min MII-pH-monitoring with postprandial phase can be used to quantitative estimate reflux accurately.

Low grade inflammation in irritable bowel syndrome patients

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Objective: Irritable bowel syndrome (IBS) is a multifactorial disease. Currently a question asked is the role of low-grade inflammation in the IBS pathophysiology. Faecal calprotectin is correlated with the importance of intestinal inflammation. The aim of the study was to characterize IBS patients with high faecal calprotectin and to phenotype this subgroup of patients.
Methods: This prospective study included all new IBS patients according to Rome III criteria. All patients realized a sampling of the faecal calprotectin by ELISA. All of them had normal low endoscopy and, if faecal calprotectin was higher than 50 μg/g, a negative sampling of the ANCA/ASCA and a normal exploration of the small bowel (capsule endoscopy, computed tomography enterography or magnetic resonance enterography). All patient underwent clinical examination and standardized tests and were proposed to have explorations with glucose and fructose breath tests, colonoscopy with colonic biopsies and rectal barostat.
Results: Ninety three patients, mean aged 43 years, 73.1% female were included. Among these patients, mainly subtype with diarrhoea (66.7%) and post infectious in 26.9%, 34 (36.6%) had an increased faecal calprotectin. In this subgroup of patient with high level of faecal calprotectin only the age tends to be older ($p = 0.06$) and calprotectin level was correlated with age ($p = 0.03$, $r = 0.22$) and with a low visceral sensitivity ($p = 0.03$, $r = 0.3799$).
Conclusion: Elevated faecal calprotectin was high in 1/3 patient IBS. They could not be identified by their clinical phenotype. Calprotectin level is even more higher than patients are older and without visceral hypersensitivity.

Effect of sacral nerve stimulation on visceral mechanosensitivity in rats

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Objective: Sacral nerve stimulation (SNS) is an alternative surgical treatment of refractory urge incontinence and/or fecal incontinence, and more recently of irritable bowel syndrome. Despite its clinical efficacy, the mechanisms of action of SNS remain poorly understood. The aim of this work was to evaluate the effect of SNS on visceral mechanosensitivity in rats with and without visceral hypersensitivity.
Methods: Anesthetized Sprague-Dawley rats were treated with SNS, performed by implanting an electrode close to the sacral nerve root S1, or sham stimulation. Colonic hypersensitivity model was obtained by non-inflammatory cross-sensitization of the colon induced by acetic acid instillation in bladder. To decipher the mechanisms underlying SNS effect, rats were administered with a non-selective opioid receptor antagonist (naloxone).Colonic mechanosensitivity was evaluated using the variation of arterial blood pressure as a spino-bulbar reflex in response to graded isobaric colorectal distension. C-fos immunoreactive neurons were quantified in spinal and supraspinal sites. Mu Opioid Receptor (MOR) internalization was counted in the sacral spinal cord with sham or effective SNS.
Results: Sacral nerve stimulation reduced blood pressure variation in response to colorectal distension in both naive and hypersensitive rats. This effect was prevented by intrathecal naloxone administration. Moreover, SNS prevented c-fos increase in the spinal cord and supraspinal sites in both models. MOR internalization was significantly higher in stimulated group.
Conclusion: Sacral nerve stimulation impacts on visceral mechano-sensitivity by decreasing the spino-bulbar reflex in response to colorectal distension in rats with and without visceral hypersensitivity. This was associated with modification of neuronal activity in the central nervous system. Spinal opioid receptors are likely implicated in this effect.

Modulation of gastrointestinal peptides by gastric electrical stimulation in patients with nausea and/or vomiting

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Objective: Gastric electrical stimulation (GES) is an alternative therapy for gastroparetic patients with nausea and/or vomiting refractory to pharmacological medications. However mechanism of action of GES remains unknown. The aim of this work was to assess the modulation of gastroduodenal peptides by GES in patients with refractory nausea and/or vomiting.
Methods: Ten patients suffering from nausea and/or vomiting refractory to prokinetics and antiemetics were included in this study. Vomiting episodes, gastrointestinal quality of life index (GIQLI) and gastric emptying were determined before (M0) then 4 months after the ON period of the blinded crossover (M4). Gastric biopsies as well as blood samples were collected at M0 and M4. Ghrelin and leptin expression was measured in gastric extracts by Multiplex analyses. Ghrelin, leptin gastric inhibitory polypeptide (GIP), glucagon-like peptide 1 (GLP-1) and peptideYY (PYY) were also quantified in blood samples using the same method.
Results: Among clinical parameters only vomiting episodes were slightly reduced by GES therapy ($p = 0.09$). Ghrelin and leptin levels in gastric extracts were not modified by GES. In blood, only PYY plasma concentration was significantly reduced ($p < 0.05$) 2 h following test meal ingestion at M4. Interestingly, plasma leptin level change between M0 and M4 was positively correlated with vomiting reduction and GI-QLI improvement.
Conclusion: Gastric electrical stimulation has not a major impact on gastroduodenal peptides expression pattern in gastroparetic patients. Plasma leptin level seems to be associated with vomiting episodes improvement.

Early life stressful events impaired enteric antimicrobial activity and triggered commensal *Escherichia coli* overgrowth responsible for visceral hypersensitivity in adult mice

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Objective: Early life stressful events induce long lasting alterations of intestinal homeostasis associated with susceptibility to develop gastrointestinal disorders at adulthood. Neonatal period is characterized by immature intestinal mucosa. For instance, Paneth cells appear only 2 weeks after birth. Our aim was to determine the consequences of neonatal maternal separation (MS) in mice on enteric antimicrobial activity and subsequent consequences on intestinal microbiota, systemic immune response toward microbiota and visceral sensitivity.
Methods: Pups were separated from their dams and the litter 3 h/day for 10 days starting from day 2. Adult female mice were studied at day 50. Visceral sensitivity in response to colorectal distension was evaluated. Cultivable aerobic and anaerobic bacteria as well as *Escherichia coli* populations were counted in feces and ileum. Specific IgG against *E. coli* were measured by ELISA in plasma. Lysozyme antimicrobial activity against peptidoglycan was dosed in feces.
Results: In 50-days old mice, MS induced a decrease of enteric antimicrobial activity associated with intestinal commensal *E. coli* overgrowth and an increase of anti-*E. coli* IgG and IgA in plasma. Furthermore, MS increased IFNγ and TNFα in ileum and induced visceral hypersensitivity in response to colorectal distension. In order to decipher whether or not those alterations were a consequence of *E. coli* overgrowth, adult mice were force fed daily with 109 commensal *E. coli* for 15 days. *E. coli* gavage reproduced intestinal *E. coli* overgrowth as well as anti-*E. coli* IgG and IgA increase and visceral hypersensitivity without modification of enteric antimicrobial defense.
Conclusion: Altogether our results highlighted that early life stressful events impair the development of antimicrobial defenses and promote commensal bacterial overgrowth leading to abnormal response toward microbiota and visceral hypersensitivity.